DEVELOPMENTAL PROPOSAL

Spatial variation in chemical defenses of Kachemak Bay macroalgal communities in correlation to abiotic factors

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ABSTRACT

Kelp beds and the communities they establish are very productive ecosystems that provide important habitat and niches for a myriad of organisms. The kelp beds in Kachemak Bay, Alaska, are undergoing changes on various spatial and temporal scales that are not clearly understood. Apart from biological factors such as grazers, it is known that abiotic factors influence kelp bed distribution and composition. It is suggested here to use kelp beds in Kachemak Bay as a case study as the north and south shores of the bay differ in their hydrographic and other environmental regimes. The goal of this study is to establish a measure of fitness for important players in kelp bed communities and analyze correlation with differing environmental factors. The measure of fitness will be the chemical defense levels of macroalgae and benthic sessile invertebrates against bacterial infection and fouling. The rational for this parameter is based on the paradigm that organisms will only be able to produce chemical defenses under environmental conditions that are optimal for growth and reproduction. Under stressful conditions such as low salinity, reduced light, high temperatures or increased sedimentation organisms have to allocate the limited resources into maintenance. It is hypothesized here that the biodiversity and chemical defense levels of kelp beds under oceanic conditions at the south shore of Kachemak Bay will be higher than those under more estuarine conditions of the north shore. Once this basic relationship is established, the effects of individual environmental factors on chemical defense production can be tested in future laboratory experiments.

BACKGROUND

Kelp beds are critical habitat in Alaska's coastal systems because of their high productivity and diversity of associated communities (Foster and Schiel 1985). Kelp has high growth rates providing food for grazers and also for the detrital food web. These complex three-dimensional habitats support tightly linked trophic webs (Mann 1973, Dayton 1985, Foster and Schiel 1985) and serve as nursery, refuge, forage and spawning sites for many associated invertebrates, fishes, birds and marine mammals (Foster and Schiel 1985, Steneck et al. 2002). Important commercial and sport fishes such as juvenile Pacific cod, Pacific halibut, and salmon also depend on these kelp beds (Abookire et al. 2001, Hamilton 2004).

Recently, several large beds in Kachemak Bay have disappeared temporarily or permanently. The *Alaria fistulosa* bed at the entrance of Jakolof Bay disappeared in the late 1970's, the *Nereocystis luetkeana* bed of the Archimandritof Shoals off the Homer Spit disappeared in 2000, but showed signs of recovery in 2002, and the Seldovia *Nereocystis luetkeana* bed at Outside Beach showed partial clearance in 2002. Kelp beds in Kachemak Bay were mapped using low altitude aerial photography between 2000 and 2002 (Schoch 2001). Spatial variability was noted in terms of presence, size and density of beds (Schoch 2001, Schoch and Chenolet 2004). The reasons and dynamics for such changes are largely unknown. Some suggested causes may be due to natural fluctuations of grazer populations (Chenelot 2003). Others suggest climate change related fluctuations in temperature, light, salinity, and nutrient regimes (Dayton et al. 1999). Turbidity and light attenuation may change due to accelerated glacial stream input and increased sedimentation rates due to headland erosion. Disturbances due to human activities such as logging, fish processing, commercial fishing, sewage waste disposal, recreation and mariculture are also possible causes.

Abiotic factors can influence kelp bed overall community structure and within this, biodiversity. Between algal stands, the main differences in community structure are supposedly due to physical effects, while within macroalgal stands biological processes have the most important effects on persistence and stability of community structure (Dayton et al. 1984). The distributional evidence of kelp beds points to geographic boundaries being imposed by physical requirements of light, temperature, and nutrients for individual species (Foster and Schiel 1986). Between-site differences in sessile invertebrates have also been attributed to environmental conditions such as sedimentation and sand burial (Ostarello 1973, Grigg 1975).

The environmental stress theory states that an organism under stressful conditions will be less able to acquire resources and will allocate a greater proportion of these reduced resources into maintenance, and less into growth, reproduction and defense (Cronin 2001). The production of chemical defenses is costly and supposedly detracts from that available for growth, reproduction, maintenance, and resource acquisition (Amsler et al. 2001, Cronin 2001). Environmental stresses including low salinity, increased temperature, reduced light, and low nutrient levels can affect concentrations of chemical defenses in marine macroalgae (Van Alstyne et al. 2001). For example, phlorotannin concentrations of *Fucus vesiculosus* and *Ascophyllum nodosum* in a Norwegian fjord were lower in algae closer to the freshwater source in the estuary (Pedersen 1984). Also the recent findings of low levels of antibacterial defenses in Arctic invertebrates from Svalbard (Lippert et al. 2003) were attributed to the estuarine conditions of the study area.

Marine organisms are virtually immersed in a "bacterial bath" (Jenkins et al. 1998), and pathogenic microbes can devastate populations of marine plants and animals (Kubanek et al. 2003). Yet, many sessile organisms such as seaweeds and sponges suffer remarkably low levels of microbial infection, despite the lack of cell-based immune systems (Kubanek et al. 2003). Marine algae, as well as other benthic organisms, are relatively free from settlement by fouling organisms due to the production of secondary metabolites with antibacterial and antifouling properties (reviewed by Bhadury and Wright 2004).

The goal of this study is to increase our understanding of the influences that environmental factors have on the biodiversity and chemical defense levels of kelp bed communities. Using antimicrobial properties as a measure of fitness in a comparison of kelp communities under differing environmental conditions will help to indicate which environmental factors, if any, are affecting kelp bed communities. The results of this developmental study will provide direction for future laboratory investigations on the effects of specific, isolated abiotic factors on algal and invertebrate fitness. This study is novel in that it will analyze a large number of species (both canopy and understory) representing entire communities, allowing interpretation of results at the community level. It will further extend the current knowledge on antimicrobial defenses in marine organisms to the northern Pacific, an understudied region to date but one that is highly exposed to the threats of environmental changes due to global climate change.

STUDY SITE:

Kachemak Bay is a 39 mile long bay contiguous to the southeastern entrance of Cook Inlet. It is divided by the 4.5 mile long Homer Spit into an inner bay and an outer bay. Cold, nutrient-rich up-welled water entering lower Cook Inlet from the Gulf of Alaska creates oceanic conditions along the south shore to the head of the bay (Burbank 1977). At the head of the bay is significant freshwater and sediment input from nine glacial streams originating from the Harding Icefield. A strong baroclinic jet develops in late summer and fall and carries warm, low salinity, and turbid waters along the north shore out into Cook Inlet (Burbank 1977, Schoch and Chenelot 2004). Three sampling sites will be established on each shore of outer Kachemak Bay: south shore possible sites include Outside Beach near Seldovia Point, outer Jakoloff Bay and Hesketh Island; possible sites on the north shore include opposite ends of the Archimandritof Shoals just off the end of the Homer Spit and the Seafair Beach. All work will be performed out of the Kasitsna Bay Marine Lab using its Cold Water Diving facilities.

The following hypotheses will be tested:

HYPOTHESIS 1: The biodiversity of kelp bed communities of the south shore of Kachemak Bay will be higher than those of the north shore.

We suggest the following objectives to test the first hypothesis:

1. To determine density of canopy kelp species and the percent cover of all other major macroalgal understory and benthic invertebrate species at study sites.
2. To determine salinity, temperature, light irradiance, sediment accumulation, abrasion effects and nutrient concentrations at the study sites.

Methods

Each site will be sampled during August at the peak of macroalgal productivity and hydrographic variation between the two shores. Using SCUBA, three 30 m transects will be randomly placed and five 1 x 1m quadrats will be taken at random locations at each transect. Within each 1 x 1m quadrat, stipe counts will be made of kelp species and all macrophytes and conspicuous macrofauna (>2 cm length) will be identified *in-situ,* counted, and an estimate of percent cover will be made (Konar and Iken 2003). Samples

of dominant macroalgal and invertebrate species will be collected for voucher specimens and for testing chemical defense levels (see hypothesis 2). Biodiversity of the two shores will be compared using the Shannon-Wiener Diversity Index and by analysis of similarity matrices (PRIMER v6).

One HOBO light intensity and temperature data logger will be placed at each site to obtain continuous readings from July through September. Surface and bottom salinity will be determined using a portable refractometer during every dive at each site.

Two sediment traps will be located randomly at each study site. Each sediment trap will consist of three polyvinyl chloride (PVC) tubes that are open at the top and closed at the bottom, attached to a central stake, which will be permanently fixed to the substratum (English et al. 1997). The traps will have a height : diameter ratio of 5, ideal for the collection of sediment in a turbulent area without resuspension of sediments within the traps (Hargrave and Burns 1979). Sediment traps will be sampled biweekly and quantified by filtering onto GF-F filters and drying at 60° C for 24 hours.

Surface and 1 meter above bottom seawater samples will be collected in polypropylene bottles for nutrient analysis. Nutrient concentrations, including nitrate, ammonia, and phosphate will be measured monthly during July through September to determine if there are any differences in the nutrient regimes among sites. Nutrient analysis will be performed by Cook Inlet Keeper (www.inletkeeper.org) using a Technicon Auto Analyzer II.

The abrasion factor due to water motion and suspended particles will be quantitatively estimated by measuring the weight loss of clod cards submerged for 14 days (may need to be adjusted) monthly at each site. The protocol for clod cards will be as in Konar (2000).

The different environmental factors will be compared between the two shores by multivariate analysis using PRIMER v6.

HYPOTHESIS 2: Sessile organisms in kelp bed communities produce chemicals with antimicrobial properties, and these chemical defense levels will be higher in organisms from south shore than north shore kelp beds.

We suggest the following objectives to test the second hypothesis:

1. To determine the chemical defense levels of canopy and understory macroalgal species and select sessile benthic invertebrate species using sympatric antibacterial assays.
2. To compare antibacterial effects of the same species from north and south shore sites, respectively.

Methods

Algae and invertebrates will be collected at all six study sites by SCUBA (see above) and immediately processed. The organisms will be blotted dry and any ephiphytes will be removed. Voucher specimens will be pressed or preserved for later species verification. Approximately 50 g algal or invertebrate tissue will be extracted three times subsequently with 1:1 CH_2-Cl_2/CH_3OH followed by three extractions with 1:1 CH_3OH/H_2O. The combined CH_2-Cl_2/CH_3OH extracts will contain non-polar compounds

and the combined CH_3OH/H_2O extracts will contain polar compounds. Where possible, 5 replicates of individual organisms per site will be used to assess variation. The crude extracts will be filtered, dried and weighed. The extract yield per g WW is determined as the natural concentration (1x) (Lippert et al. 2003) and 10x, 1x and 0.1x concentrations will be used in antibacterial assays.

For bacterial assays, at least five bacterial strains will be isolated from stones, seawater, and sediments collected with SCUBA at the study sites following the protocol of Lippert et al. (2003). Bacteria will be obtained from stones by swabbing the surfaces with sterile cotton tips followed by inoculation of Zobell marine agar plates. 100 µl seawater samples will be used for inoculation of agar plates. Sediments will be suspended in 10 times volume of sterile seawater, allowed to settle and 100 µl of the supernatant will be used for inoculation of agar plates. Also, marine fouling bacteria will be swabbed from aluminum test panels deployed for 7 days at the sites (Sonak and Bhosle 1995). The bacterial strains will be identified by sequence analysis of 16S rDNA or by fatty acid methyl ester analysis (Microbial ID, Newark, Del, using Sherlock Microbial ID system).

Antimicrobial activity will be evaluated using the agar diffusion technique (Cole 1994). These assays are used to establish a dose response curve for a given bacterial strain against a given compound or extract. Active extracts applied to paper discs prevent bacterial growth on agar plates and the size of inhibition zones around the discs is a relative measure of activity strength. Bacterial strains will be grown in Zobell marine broth at room temperature overnight prior to the experiments. Innocula of 200 µl - 500 µl of each strain will be spread evenly on separate Zobell marine agar plates. 10 µl of crude extract of the various concentrations will be applied to each side of a sterile paper disc (6mm, Whatman) and allowed to dry. Control treatments will consist of solvents only. Discs will be placed on inoculated agar discs (replicate extracts on different plates) and bacteria will be allowed to grow for at least 24h. After incubation the growth inhibition zones around the paper disks will be measured to the nearest 0.1 mm. At least 5 replicates of each extract type (polar and nonpolar) of individual extractions will be tested per bacterial strain.

All data will be tested for normality (Kolmogorov-Smirnov) and subjected to Bartlett's test for homogeneity of variance groups. One way analysis of variance (ANOVA) will be applied to compare antibiotic activity between the same species of the north and south shore sites.

TIMELINE:

Date	Activity
June	Site surveys, monitor abiotic factors, pilot clod card and sediment trap experiments, isolate marine bacteria, conduct trial antibacterial assays
July-Sept	Monitor abiotic factors, conduct clod card abrasion studies, conduct sediment trap studies, isolate bacteria
August	Conduct biodiversity transects, collect organisms for extraction
Sept-Dec	Conduct antibacterial assays, analyze data, prepare report

BUDGET JUSTIFICATION

This proposal seeks funding for one summer season of the described studies and will contribute to the progress of a Doctor of Philosophy degree for Tania Spurkland at the University of Alaska Fairbanks. An AAUS scholarship would be utilized to obtain necessary scuba diving equipment. The remainder of the funding has been sought through the Cold Water Dive Program of the National Undersea Research Program and Alaska Sea Grant. A summer stipend for TS is requested. Funds are also requested to cover laboratory fees at the Kasitsna Bay Laboratory and the use of small boats for transportation to and from sample sites. No housing at the lab is requested since TS is living in Seldovia. No equipment support is requested. All necessary equipment is provided through the UAF School of Fisheries and Ocean Science laboratories. Support is requested for supplies including chemicals for extractions, bacterial assays, HOBO temperature and light data loggers (3 of each already available, 3 more requested to have one per site), and materials for sediment traps and clod card abrasion studies. Funds are also requested for marine bacterial identification and nutrient analyses. A Graduate School Fellowship has been awarded to TS for the 2005/2006 academic year and will cover stipend after August 2005.

Project Budget		
	Subtotal	**Total**
Salary		
Spurkland (summer)	$4,832	
TOTAL SALARY		**$4,832**
Travel		
Spurkland: Homer-Fairbanks (1200 miles @ $2/gallon)	$75	
(round trip)		
Water taxis: Homer-Seldovia 4 @ $50	$200	
TOTAL TRAVEL		**$275**
Services		
Rubber skiff: 19 days @ $30/day	$570	
21' whaler w/ operator: 19 days @ $50/1/2 day	$950	
Dive locker: 76 dyas @ $10/day	$760	
Small Lab: 24 days @ $50/day	$1,200	
Nutrient analysis	$540	
Marine bacteria identification	$385	
TOTAL SERVICES		**$5,405**
Supplies		
SCUBA equipment		
Dive compass	$200	

Buoyancy compensator	$500	
Regulator	$400	
Dive computer	$500	
Chemicals	$500	
Bacterial assay supplies	$750	
HOBO temp and light loggers	$1,362	
Sediment trap/clod card materials	$200	
Misc. supplies	$200	
TOTAL SUPPLIES		**$4,612**
Subtotal	$15,124	
TOTAL FUNDING REQUESTED		**$15,124**

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